REMARKS

Claims 15 – 23, and 25 – 27 were rejected under 35 U.S.C. 103(a) as being unpatentable over WO 94/18992(McCormick) in view of Raj et al.

Applicants respectfully submit that this rejection should be withdrawn for the following reasons.

Attached hereto please find a Declaration of Dr. William Kaelin (also referred to as the "Kaelin Declaration") (an unexecuted copy attached hereto), a co-inventor of the above-identified application, addressing the cited art. Dr. Kaelin states that he is familiar with the above-cited references and does not believe that these references in any way teach or suggest the claimed method of selectively expressing a gene in a malignant cell, by determining whether the malignant cell expresses sufficient E2F to cause expression of a gene operably linked to an E2F responsive promoter (Kaelin Declaration ¶¶ 3-5). Dr. Kaelin goes on to state:

> Rather, these references consider the role of E2F as a simple off/on trigger for gene expression. It was known that $_p$RB negatively regulates E2F and that this inhibition is relieved (under physiological conditions) by phosphorylation of $_p$rB which occurs at or near the time cells enter S-phase (See Figure 1). For example, this is shown in Raj at page 279, second column, where it states:
>
> > The activity of E2F1 appears to be regulated by its interaction with various cellular proteins, including pRb. pRb binds directly to E2F1 and inhibits its transactivation (Chellapan et al, 1991; Helin et al., 1993; Hiebert et al., 1992; Weintraub et al., 1992). In addition to pRb, E2F1 also forms complexes with p107, cyclins A and E, and cyclin dependent kinases (cdk). The **association of E2F1** with these various cellular proteins **appears to be cell-cycle regulated**, with the appearance of distinct complexes at precise states of the cell cycle (Nevins, 1994). [emphasis added] (Kaelin Declaration ¶ 5.)

Dr. Kaelin concludes that Raj is talking about a binary system, with E2F either "on" (when not bound to $_p$RB) or "off" (when bound to $_p$RB) (Kaelin Declaration ¶ 6).

Dr. Kaelin adds that "Raj compared HJC-15 hamster glioma cells and U-87 MG human glioma cells (Kaelin Declaration ¶ 7)."

Dr. Kaelin further adds:

> Moreover, in the hamster glioma cells, Raj found a protein complex that would bind to E2F binding sites. The protein was not identified as any E2F species but rather completely separate proteins referred to Glial E2F1 – associated proteins (GEAPs) having a distinct molecular mass.
>
> Although Raj showed that as expected E2F1 would activate E2F-responsive promoters in U-87 MG cell, E2F1 decreased the activity of the promoters in HJC-15.
>
> **Consequently, Raj provides absolutely no basis for looking at levels of free "E2F."** (Kaelin Declaration ¶¶ 8-10, emphasis added.)

Applicant respectfully submits that WO 94/18992 (McCormick), either alone or in combination with Raj, does not teach or suggest the claimed invention.

As stated by Dr. Kaelin:

> Over time it was proposed that E2F-responsive promoters could be viewed as being in one of three states: (1) fully activated by free E2F, (2) fully repressed by pRB/E2F, or (3) in a basal state (where transcription was driven by non-E2F transcription factors) (See Figure 2). In proliferating cells, pRB would be phosphorylated and E2F-responsive promoters activated/derepressed. In quiescent or resting cells, pRB would be unphosphorylated and E2F-responsive promoters repressed.
>
> Multiple groups prior to 1997 showed, in cell culture experiments, that **E2F-responsive promoters** were **more active in proliferating non-transformed cells than quiescent cells** and that mutation of E2F sites typically led to derepression under resting conditions. For example, several groups showed that the promoter for E2F1 itself, which contains 4 E2F sites, was serum-inducible in non-transformed cells such as NIH3T3 cells and REF-52 cells [cites ommited]. THUS, PRIOR TO OUR WORK, THE EXPECTATION WOULD BE THAT E2F-RESPONSIVE PROMOTERS WOULD BE ACTIVATED IN RAPIDLY PROLIFERATING NORMAL CELLS AS WELL AS IN TUMOR CELLS. (Kaelin Declaration ¶¶ 12-13, emphasis in original)

As explained by Dr. Kaelin in paragraphs 14 and 15 of his Declaration, "The surprising finding we made was that while the E2F1 promoter was very active in tumor cells in vivo, its activity could not be measured in rapidly dividing hepatocytes, which are normal cells" (Kaelin Declaration ¶ 14). Based upon the state of the art at the time, one would have expected that the E2F responsive promoters in such proliferating normal cells would have been activated. However, Applicants were using a sensitive reporter system to detect expression from E2F responsive promoters. Using the "suicide protein," viral thymidine kinase, as the reporter, applicants confirmed their surprising discovery that tumor cells had much higher expression levels than the rapidly dividing hepatocytes, and in fact saw virtually no expression in the normal cells. "Our unexpected findings appear to be at least partly due to the establishment of a positive feedback loop in tumor cells" (Kaelin Declaration ¶ 15). This type of selective expression simply was never suggested by the prior art.

In paragraph 16 of his Declaration, Dr. Kaelin concludes:

> Accordingly, we taught there was a selective expression in these cells, and that to determine if one would see such an "activated" and thus selective expression, it is important to determine that there is a sufficient amount of E2F present in the cells to result in such a selective expression. **There is simply nothing in these references that in any way suggests or motivates one to look at levels of E2F expression** [emphasis added].

The work of the present inventors has been acknowledged and replicated (Kaelin Declaration ¶ 17). See paragraphs 17 of the Kaelin Declaration for a discussion of Jakubczak, J.L., et al., Cancer Research 63:1490-1499 (2003). Jakubczak et al at page 1498 confirms the teaching of the present invention (Kaelin Declaration ¶ 18) concluding:

> In conclusion, we have demonstrated that the E2F vector selectively kills a broad range of Rb pathway-defective tumor cells *versus* normal cells. We have shown that the mechanism of this selectively is based on the presence of E2F binding sites within the E2F-1 promoter in the virus and also a disruption of the Rb pathway in the target cell. This characteristic will allow therapeutic broad application of this vector to many cancer types. We also show that the ability of the vectors to replicate is a requirement for full oncolytic activity both *in vitro* and *in vivo*. With

> systemic delivery, this vector is less toxic than wt and Add*l*1520, additionally indicating its selectivity. Most importantly, we have demonstrated potent antitumor efficacy *in vivo* that is greater than that of Add*l*1520.

In paragraph 19 of his Declaration Dr. Kaelin concludes:

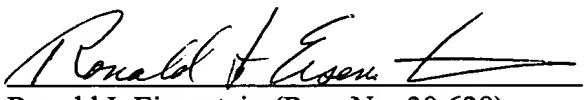
> Accordingly, I respectfully believe that such tumor selectivity, and the importance of looking for E2F expression in malignant cells, is in no way suggested by the prior art references.

Accordingly, this rejection of the claims should be withdrawn.

Applicants respectfully submit that the reasons for withdrawing the rejection of the above claims is equally applicable here and they incorporate those arguments herein. The further addition of the `045 for showing that cytotoxins were known, in no way suggests their applicability with other references. Indeed, without the knowledge from Applicants that one could selectively express such genes in a malignant but not a normal cell the skilled artisan would be concerned that there would be substantial harm to normal cells resulting from the use of such cytotoxins. Accordingly, Applicants respectfully submit that this rejection should be withdrawn for that additional reason.

In view of the foregoing applicants respectfully submit that all claims are in condition for allowance. Early and favorable action is requested.

Respectfully submitted,



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